REMARKS

With the entry of the present amendment, claims 1-21, 23-27, 29-35 and 43-48 are in this application. Claims 1-3 have been amended to better claim the invention. The amendments to claim 1 and new claims 47-48 are supported by as-filed Specification at Figure 2, and at page 11, first full paragraph, for example, and by the as-filed claims. None of the amendments made herein constitutes the addition of new matter.

The Rejections under 35 U.S.C. 102

Claims 1-4, 6-16, 18-21, 23-24 and 29-30 have been rejected under 35 U.S.C. 102(b) as allegedly unpatentable over Heinrich et al. (2000) PNAS 97:8229-8232. Applicants respectfully traverse this rejection.

Heinrich is said to teach a tetracycline repressible female-specific lethal genetic system in *Drosophila melanogaster*. One component is the tetracycline-controlled transactivator gene under the control of the fat body and female-specific transcription enhancer from the yolk protein (*yp1*) gene. The other component consists of the proapoptotic gene hid under the control of a tetracycline-responsive element. Males and females of a strain carrying both components are viable on medium supplemented with tetracycline, but only males survive on normal medium. Heinrich is said to teach the expression of tTA controlled with the female and fat body specific transcription enhancer from the *yp1* gene. Heinrich is further said to teach the *yp1* enhancer upstream of the *hsp70* minimal promoter used to drive expression of the tTA coding sequence and that in the absence of tetracycline, tTA binds to *tetO* and induced expression of the proapoptotic gene *hid*. The loss of the fat body results, with female specific lethality, and because ectopic expression of the proapoptotic gene *hid* can lead to tTA, which is inactive in the presence of tetracycline, expression of tTA is controlled with the female specific enhancer of *yp1*. Heinrich is further said to teach because components of the system are either conserved (yolk protein genes) or known to function in both Drosophila and mammalian cells, the system could be used to make genetic sexing strains for a variety of insect pests. Heinrich is said to teach the system was designed such that female flies would die in the absence of tetracycline due to

widespread cell death in the fat body, expression of tTA is controlled by the female and fat body specific *yp1* enhancer, binding of tTA to tetO results in inactivation of expression of the proapoptotic gene *hid* and induction of apoptosis in fat body results in female lethality because the fat body is an important tissue for metabolism and food storage in insects. The amount of induced cell death is said to be very sensitive to the level of ectopic *hid* expression which in the female lethal system depends directly on the level of tTA expression. It is said that position effects could be minimized b bracketing the *yp1*-tTA and tetO-*hid* constructs with insulator elements. Heinrich teaches the effect of diet on female lethality, consistent with prior studies that showed the *yp1* enhance is responsive to diet, especially yeast and that it would be of interest to determine whether the diet response is mediated by either the sex-specific double sex protein or the proteins that bind the b-zip or we sites of the enhancer.

Applicants provide the following discussion to clarify the salient and unique features of the present invention. The key aspect of the invention is a system which employs positive feedback to control gene expression. In any kind of feedback system, for instance audio feedback, an output signal plays into the input. In the present biological system, the expression product of the control factor gene (for instance tTA in certain embodiments could be considered analogous to the “output: of the first element. Once expressed, tTA then induces expression from the second element. This is, at least in part, known from the art, including Heinrich as the Examiner has correctly pointed out.

However, Applicant respectfully point out that what is new in the present claimed invention is that the tTA expression product also takes part in its own positive feedback loop. The first element comprises the control factor gene and a promoter for same. Notably, the claim requires that the expression product of the control factor gene of the first element serves as a appositive transcriptional control factor for both the at least one fist promoter in said first element. That is to say, the tTA expression product acts on its own promoter in the first element so that the more tTA that is expression the greater the action on the tTA promoter to drive expression of yet more tTA from the first element. Thus using the sound system analogy, the tTA output is played (or fed) back into the input in the first element by effectively driving its own expression (in the absence of

tetracycline). This is the positive feedback loop at the heart of the invention.

At the same time, the expressed tTA in the absence of tetracycline) is also driving expression from the second element. Therefore, the key words to highlight to the Examiner in the main claim are:

wherein an **expression** product of the control factor gene **of the first element** serves as a positive transcriptional control factor for **both**:

(i) **the at least one first promoter in said first element**; and

(ii) the at least one second promoter in said second element.

For the purposes of further explanation, the use of figures may be helpful and this is best explained with reference to certain preferred embodiments, such as claim 4. In such a system, the first and second elements comprise the tetO enhancer (the claim also specifying that the control factor gene product is the preferred tTA or a variant). Thus, the system of claim 3 would look like:

First element:

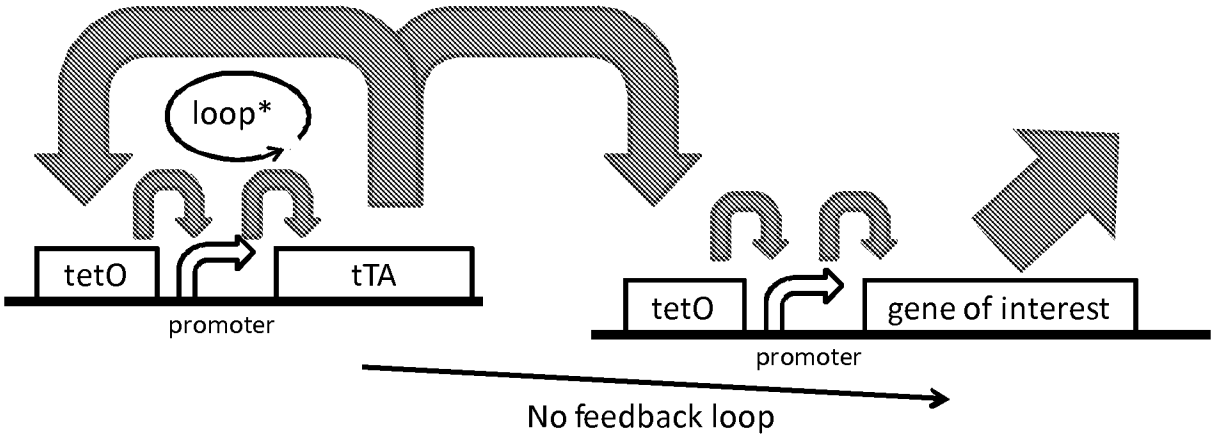
[tetO] [suitable promoter] – [tTA or variant]

Second element:

[tetO] [suitable promoter] - [gene of interest]

By way of explanation, these 'suitable promoters' are *typically* minimal promoters. Minimal promoters, as mentioned in the specification are promoter elements which can respond to an enhancer (for instance tetO with tTA bound to it), but do not drive much expression on their own (or with tetO adjacent but no tTA). This is by no means an essential feature (i.e. other types of promoter can be used), but it is the simplest case to consider.

The two elements of the invention work together in the following way:



This is analogous to Figure 2 of the present application, but presented as two elements, according to line 21 of page 11 of the PCT specification ("Alternatively,….). The key part here is the positive feedback loop shown by the arrow marked by a star (*). This feedback is missing from Heinrich. Heinrich has two constructs.

In Heinrich, the first construct has:

[Yp1enhancer] – [hsp70 minimal promoter] – [tTA]

The second construct in Heinrich has:

[tetO] - [hsp70 minimal promoter] - [hid]

This is clear from the Methods section and is helpfully illustrated diagrammatically in their Figure 1 (page 8230), reproduced below.

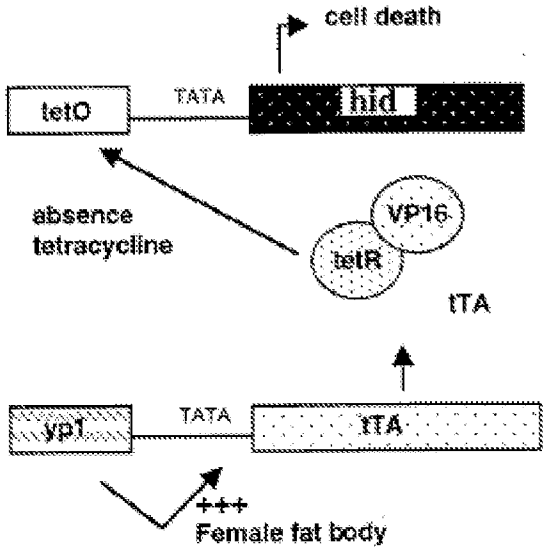


Fig. 1. The tetracycline-regulated female-killing system. Expression of tTA is controlled with the female- and fat-body-specific transcription enhancer from the *yp1* gene (18). In the absence of tetracycline, tTA binds to tetO and induces expression of the proapoptotic gene *hid*. The loss of fat body results in female-specific lethality. In the presence of tetracycline, females are fully viable, because the binding of tTA to tetO is inhibited, switching off *hid* expression.

This is the classic configuration of the tet-off expression system, with no hint of positive feedback. It is apparent that in Heinrich's first construct, the expression of tTA is driven by the **Yp1 enhancer** (acting on an hsp70 minimal promoter). However, it is clear in the reference that there is nothing in Heinrich's first construct that is responsive to tTA.

The tetO enhancer is responsive to tTA, but this is only found in the second element of Heinrich. The Yp1 enhancer of the first of Heinrich's constructs is certainly not responsive to tTA. Therefore, requirement (i) of Applicants' claim is not disclosed in Heinrich. In other words, there is no positive feedback in Heinrich's first element as the expression product of the control factor gene only acts to drive transcription from the second element and not from both the first and second elements.

Applicants respectfully note that an apparent misunderstanding of the teachings of the cited Heinrich reference appears at page 6 of the office action, in the summary of our previous arguments. The Patent Office fails to state that tTA acts as a positive transcriptional control factor (i.e. drives expression) of Heinrich's first element and only focuses on tTA's action on Heinrich's second element.

Thus, Applicants respectfully emphasize that tTA does not act to drive expression from Heinrich's first element. Thus, Heinrich fails to disclose the necessary positive feedback (of tTA on its own expression) according to the present claimed invention.

Another version of the same apparent Patent Office misunderstanding is found on Page 3, line 7-8, of the office action and again where that block of text is repeated on page 7 (line 14-15):

> "Heinrich teaches the two component system with a positive control factor tTA which controls expression of **both** components…" [emphasis added]

As explained above, in Heinrich, tTA does not drive expression of both, but of only one element (the second element). Thus, the main claim is novel over Heinrich for this reason alone.

A further point of distinction over Heinrich is that Heinrich does not teach "on the same construct", contrary to the Examiner's comments. All of Heinrich's constructs have either Yp1-tTA **or** tetO-hid, but not both. Thus, the main claim is also novel over Heinrich by virtue of this distinction.

In the interest of advancing prosecution and without acquiescing to the rejection, Applicants have amended claim 1 to specify a two component system with a positive control factor which controls expression of both components. By contrast, the two components of the Heinrich system are separately controlled – tTA by the *yp1* genetic sequences and the *hid* coding sequence is expressed on the regulatory control of tetracycline responsive genetic sequences (see Figure 1, for example).

In view of the distinctions over Heinrich presented herein, Applicants respectfully submit that the claimed invention is not anticipated by the cited Heinrich reference, and the withdrawal of the rejection is respectfully requested.

<u>The Rejections under 35 U.S.C. 103</u>

Claims 1-16, 18-21, 23-24 and 29-30 remain rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Heinrich et al. (2000) PNAS 97:8229-8232 in view of

Savakis (US 2003/0150007) and Loukeris (1995) PNAS 92:9485-9489 Applicants respectfully traverse this rejection.

Heinrich is said to teach a tetracycline repressible female-specific lethal genetic system in Drosophila, as discussed in detail above.

Savakis is characterized as teaching, at the time of the present invention, the use of a modified transposon, wherein the modification includes removal or disruption of transposase sequences or the incorporation of heterologous coding sequence(s) and/or expression control sequences. The Patent Office acknowledges that Savakis teaches a particular transposon (Minos) to generate transgenic animals and says that Savakis embraces the idea of any transposon and contemplates sequences heterologous to the species. A variety of promoters were disclosed. Savakis is also said to contemplate modified codon usage.

Loukeris is said to teach that efforts for Drosophila germ line transformation are unsuccessful because P elements of *Drosophila melanogaster* do not function in *D. hawaiensis*. Loukeris is said to teach an approach which is to use P elements from species distant to Drosophila.

Applicants have previously amended claim 1 to specify a two component system with a positive control factor which controls expression of **both** components. By contrast, the two components of the Heinrich system are separately controlled – tTA by the *yp1* genetic sequences and the *hid* coding sequence is expressed on the regulatory control of tetracycline responsive genetic sequences (see Figure 1, for example). Note that in certain claims, the regulation of gene expression is via the combination of a positive feedback loop and tissue specific or stage specific sequences.

Savakis does not suggest the system of the present invention as currently claimed, with a single regulatory factor controlling expression of both itself and a second component of the system. Different applications of genetic modifications are taught by Savakis and Loukeris.

With regard to inventive step, the Examiner largely repeats his comments on Heinrich. Applicants have provided an extensive discussion of the cited Heinrich reference above. As Heinrich is completely silent on any form of feedback, it certainly would not have been obvious to introduce this into Heinrich's system by replacing, for

instance, the Yp1 enhancer of Heinrich's first construct with one responsive to tTA, to provide the required "positive transcriptional control factor for (both) (i) the at least one first promoter in said first element" in claim 1.

In the interest of advancing prosecution and without acquiescing to the rejection, Applicants previously amended claim 1 to specific a two component system with a positive control factor which controls expression of both components of the system. This is not taught or suggested by the cited references, alone or in combination; it represents a very different strategy for regulated gene expression than is taught by the cited references, alone or in combination. This key feature does not appear to be found in the cited art. Thus, Applicants respectfully maintain that the invention as currently claimed neither taught nor suggested by the prior art, and it would not have been obvious to one of ordinary skill in the art to construct a system where the same factor controls the expression of both the regulatory factor and the second component of the system.

Moreover, please note that in the present claims, there are embodiments where the lethal gene and the regulatory factor are the same, for example tTA. The cited art does not suggest that this could be so, and with so much information in the field teaching the use of a gene heterologous to the regulatory sequences for lethality or sterility, it is clear that this is not where the art leads one of ordinary skill in the art in seeking a solution for this technical problem. It is by this self-action (autoregulation) that positive feedback in the insects is obtained in the present invention. However, the cited art lack this essential feature. Accordingly, the present inventors have established a new and nonobvious system which can be highly effective in a very wide range of insects. Thus, the present invention has considerable advantages over the prior art.

The Examiner is referred to the introduction of the present specification which states that "very few promoters or other control elements have been characterized and there remains a pressing need for such elements." This is, of course, in reference to insects. Therefore, the present inventors have established a novel system can be highly effective in insects, whereas it was not previously thought that this was possible. Indeed, our system is the equivalent to a reliable strong promoter which functions in a very wide range of insects. A single promoter with this level of expression efficacy was

not previously available. Thus, the present invention has considerable advantages over the cited prior art.

Despite the evident need for such a system, there is no mention of a controllable, positive feedback element in the cited art or any instructions as to how the skilled person may obtain one. Thus, there is nothing in the prior art to motivate the skilled person to provide a system according to the present invention which comprises a positive feedback loop, as neither of these documents suggest why this might be useful, let alone how to provide it. In other words, the cited art, despite disclosing the tetracycline/tTA system, teach completely different pest control approaches to that of the present invention.

A further advantage of the present invention is that the complete expression system can be introduced with only a single transformation event. This also means that insects homozygous for the system are homozygous at only one locus rather than two, which makes them easier to construct by breeding, and tends to reduce the fitness cost due to insertional mutagenesis.

Accordingly, not only does the present invention provide a promoter with a broad specificity throughout insects, but it also overcomes several problems that tend to occur with expression systems in the field, i.e. in actual insect populations. Thus, the present invention is not obvious over the cited art.

In view of the amendments to the claims and the discussion provided herein, Applicants respectfully submit that the claims are not properly deemed obvious over the cited art and the rejection should be withdrawn.

Claims 1, 17 and 25-27 remain rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Heinrich et al. (2000) PNAS 97:8229-8232 in view of Bessereau (2000 (WO 00/073510), Savakis et al. (EP 0955364), Horn et al. (2003). Applicants respectfully traverse this rejection.

Heinrich is said to teach a tetracycline repressible female-specific lethal genetic system in Drosophila, as discussed above, and the teachings of Savakis have been discussed above.

The cited Bessereau reference relates to genetic modifications in the nematode *Caenorhabditis elegans*, especially using transposable elements to make transgenic

nematode. There is no teaching of a two component genetic system with two components controlled by a single regulatory factor, as is now claimed.

The cited Savakis reference relates to transposons and their use in creating transgenic organisms, but it does not teach a two component genetic system with two components controlled by a single regulatory factor, as is now claimed.

Applicants have discussed the Heinrich, Savakis and Loukeris references above, and that discussion is applied here as well. It is important to note that Heinrich neither teaches nor suggests an expression control system such as that taught in the present application, wherein a transcriptional control protein positively controls its own expression and the expression of a gene of interest.

The cited Horn reference relates to genetic modification for embryo lethality in certain insect pests. Blastoderm specific promoters are used to control expression of the lethality sequences so as to achieve embryo-specific killing. There is no teaching or suggestion of a common regulatory factor to control the expression of both the regulatory factor and the lethality factor.

Applicants have discussed the Heinrich, Savakis and Loukeris references above, and that discussion is applied here as well.

Moreover, Applicants respectfully emphasize that in the present claims, there are embodiments where the lethal gene and the regulatory factor are the same, for example tTA. The cited art does not suggest that this could be so, and with so much information in the field teaching the use of a gene heterologous to the regulatory sequences for lethality or sterility, it is clear that this is not where the art leads one of ordinary skill in the art in seeking a solution for this technical problem. It is by this self-action (autoregulation) that positive feedback in the insects is obtained in the present invention. However, the cited art lack this essential feature. Accordingly, the present inventors have established a new and nonobvious system which can be highly effective in a very wide range of insects. Thus, the present invention has considerable advantages over the prior art and significant differences from the cited prior art.

Despite the evident need for such a system, there is no mention of a controllable, positive feedback element in the cited art or any instructions as to how the skilled person may obtain one. Thus, there is nothing in the prior art to motivate the skilled

person to provide a system according to the present invention which comprises a positive feedback loop, as neither of these documents suggest why this might be useful, let alone how to provide it. In other words, the cited art, despite disclosing the tetracycline/tTA system, teach completely different pest control approaches to that of the present invention.

In view of the foregoing and the amendments to the claims, Applicants respectfully maintain that the invention is not *prima facie* obvious over the cited art, and the withdrawal of the rejection is respectfully requested.

Claims 33-35 remain rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Heinrich et al. (2000) PNAS 97:8229-8232 in view of Horn et al. (2003) and Horn et al. (2000). Applicants respectfully traverse this rejection.

The Patent Office has stated that the characterization of the Heinrich and Horn (2003) references are applied here as well and conceded that these two references do not teach an expression marker. The Horn (2000) reference is said to teach the use of the fluorescent marker for Drosophila transgenesis, and the Patent Office has concluded that one of ordinary skill in the art at the time the invention was made would have been motivated to use a marker to select transgenic organisms at difference stages of development with a reasonable probability of success.

As discussed above at length, the claims have been amended to specify the two component system with the expression product of one component controlling both its own expression levels and that of the second component. This is a significant departure from the strategies of the prior art. Note also that claim 1 (base for the vector of claim 33) does not specifically recite an expression marker. The present invention as claimed, with its particular expression system and regulatory strategy constitutes a significant departure from and advance over the prior art.

In view of the foregoing discussion and the amendments to the claims, Applicants respectfully maintain hat the present invention as claimed is not *prima facie* obvious over the cited art and the rejection should be withdrawn.

Conclusion

In view of the foregoing, it is submitted that this case is in condition for allowance, and passage to issuance is respectfully requested.

If there are any outstanding issues related to patentability or with respect to this response, the courtesy of a telephone interview is requested, and the Examiner is invited to call to arrange a mutually convenient time.

This response is accompanied by a Request for Continued Examination, a Petition for Extension of Time (one month) and payment of $405.00 as required by 37 C.F.R. 1.17(e) and $65.00 as required by 37 C.F.R. 1.17(a). Two new dependent claims have been presented, but in view of the cancellation nine claims in the prior Amendment, it is believed that this amendment does not necessitate the payment of any additional claims fees under 37 C.F.R. 1.16. If the amount submitted or the extension requested is incorrect, however, please charge any deficiency or credit any overpayment to Deposit Account No. 07-1969.

Respectfully submitted,

/donnamferber/

Donna M. Ferber
Reg. No. 33,878

GREENLEE, WINNER AND SULLIVAN, P.C.
4875 Pearl East Circle, Suite 200
Boulder, CO 80301
Telephone (303) 499-8080
Facsimile: (303) 499-8089
Email: usptomail@greenwin.com
Attorney Docket No.: 7-06